July 13, 2004

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM NO. 580.16

SUBJECT: Procedures for investigations and surveillance of targeted cattle for bovine spongiform encephalopathy (BSE)

TO: Regional Directors, VS
Area Veterinarians In Charge (AVICs)

I. PURPOSE

Testing animals in the targeted populations as described in this memorandum comprises the national surveillance program for BSE, beginning in June 2004. The goal of the program is to test as many animals as possible in the described targeted high-risk population over a 12- to 18-month period. At the end of this period, the results will be evaluated and future policies will be adjusted as necessary depending on this evaluation.

The purpose of this memorandum is to clarify the procedures for:

- Foreign Animal Disease (FAD) investigations of adult cattle with clinical signs of BSE (highly suspicious for BSE);
- Sampling targeted high-risk cattle for BSE surveillance purposes (including cattle condemned on antemortem inspection); and
- Sampling limited numbers of apparently normal adult animals presented at slaughter.

II. CANCELLATION

Veterinary Services Memorandum No. 580.16, dated June 11, 1997, is hereby canceled.

III. FAD INVESTIGATIONS OF CATTLE HIGHLY SUSPICIOUS FOR BSE

Cattle exhibiting clinical signs as described below should be categorized as highly suspicious for BSE and should receive a complete FAD investigation as described in Veterinary Services Memorandum 580.4. Other animals that have abnormal central nervous system (CNS) clinical signs but do not fall into the highly suspicious for BSE category should be sampled within the established framework of the BSE Surveillance plan, and additional information on these animals should be captured in the supplemental data forms as attached.

There are no clinical signs that are pathognomonic for BSE. Animals that are highly suspicious for BSE may only display a few of the signs and signs may vary significantly in severity. True rabies suspects (*i.e.*, animals in areas where there is a significant level

of endemic wildlife rabies and where veterinary practitioners frequently encounter domestic animal exposures) should be treated as such and submitted for rabies testing prior to submission for BSE testing if they are negative for rabies.

The Office International des Epizooties Code (Appendix 3) has established a minimum number of annual investigations of cattle showing clinical signs consistent with BSE required for effective surveillance according to the total cattle population over 30 months of age. Assuming an adult cattle population of 45,000,000 for the United States, the minimum number under this guideline is approximately 450. Animals that meet the following description will be counted in this category.

The following are the criteria for identifying an animal that is highly suspicious for BSE.
Cattle of any age:

1. Affected by illnesses that are refractory to treatment, including but not limited to, anorexia; loss of condition in spite of a good appetite; pneumonia; and decreased milk yield AND that are displaying progressive behavioral changes, including but not limited to, apprehension; nervousness; excitability; aggression towards other cattle or humans; head shy with head held low; persistent kicking when milked; high stepping; difficulty in rising; excessive nose scratching; changes in herd hierarchical status; hesitation at doors, gates, and barriers; and reluctance to cross concrete or other "slippery" surfaces. Reports of progressive behavioral changes by a farmer or animal caretaker should be taken seriously even if the animal seems "normal" on investigation since in the early clinical stages of BSE these signs may be subtle and not easily noticeable by the investigating veterinarian.
2. Displaying progressive neurological signs that cannot be attributed to infectious illness and that are not responsive to treatment.

Sample collection and submission for cattle that are highly suspicious for BSE:
Cattle that show highly suspicious clinical signs should be observed over a period of time (at least 2 weeks) if possible, to determine whether the signs become progressively more severe. If at this time improvement or recovery has not taken place, the suspect animal should be humanely euthanized with an appropriate method and submitted for testing.

The following collection procedure will provide acceptable brain specimens for testing both for rabies and BSE. Submission of brain specimens for rabies testing will be left to the professional judgment of the Veterinary Medical Officer. Rabies testing should be done at the appropriate State or local public health department. Therefore, we recommend that you work with appropriate public health personnel (laboratory and epidemiology) in your State to discuss: (1) collection procedures that will provide acceptable brain specimens for testing for both rabies and BSE, (2) procedures for rabies sample submission, and (3) if rabies negative, maintenance and submission of fresh specimen quality for forwarding to the National Veterinary Services Laboratories (NVSL) for BSE testing.

The following collection procedure will provide acceptable brain specimens for testing both for rabies and BSE.

1. The entire brain should be removed intact with a portion of the cranial cervical spinal cord attached.
2. If rabies testing is required, the brain should be submitted intact to the local rabies laboratory for testing as required by the local laboratory for bilateral sampling. If appropriate and agreed upon in advance, the medulla can be removed at the level of the obex as described below. The obex and cranial cervical spinal cord can be submitted to NVSL for BSE sampling, and the remainder of the brain can be submitted to the rabies laboratory.
3. If rabies negative:
    a. The brain stem is transected at the level of the medulla (caudal to the cerebellar peduncles at cranial nerve 10) and at the junction of the medulla and spinal cord. This part of the brain stem containing the obex is placed in a screw top tube (if the carcass is retained and a fast turn around time is needed) or in a screw top bottle containing at least 200 ml of 10% neutral buffered formalin. The portion of the cranial cervical spinal cord (approximately 1/2 inch section) is placed in the plastic bag and frozen. This is the MINIMUM sample required for BSE testing and must be sent to NVSL.

    b. The reminder of the brain can be divided in half by cutting along the midline in the space between the cerebral hemispheres and continuing the midline cut through the cerebellum and the remainder of the caudal brain stem (pons and medulla). One of the cerebral hemispheres with attached midbrain should be placed in a whirl pack or plastic bag and frozen. The other half of the cerebrum with attached midbrain should be placed in a liter bottle of 10% neutral buffered formalin. The fixed samples are sent to NVSL, and the frozen samples should be held at the submitting laboratory until initial testing is completed.

Specimens should be shipped to NVSL, 1800 Dayton Road, Ames, Iowa, 50010, by Federal Express using procedures specified in Veterinary Services Memorandum No. 580.4, Procedures for Investigating a Suspected Foreign Animal Disease (FAD). The package must be marked for Saturday delivery if shipping via Federal Express on Friday.

IV. SAMPLING TARGETED HIGH-RISK CATTLE FOR SURVEILLANCE:

Animals in any of the following categories are to be included in the targeted surveillance efforts. Animals that are highly suspicious for BSE as outlined in the previous section should be treated as an FAD investigation, and follow procedures in Veterinary Services Memorandum No. 580.4. All other animals in the listed categories should be sampled as part of routine targeted surveillance. If there is a question about whether an animal should be sampled as part of routine targeted surveillance, take the sample, record the appropriate data, and submit the sample.

Definition – Targeted Cattle Population

Age – Unless otherwise designated, samples should only be obtained from animals over 30 months as evidenced by the eruption of at least one of the second set of permanent incisors.

Clinical Presentation Criteria

1. Cattle that cannot rise from a recumbent position (downer) or that cannot walk including, but not limited to, those with broken appendages; severed tendons or ligaments; nerve paralysis; fractured vertebral columns; or metabolic conditions as well as cattle that are severely weakened though they may be able to stand and walk for brief periods of time.

2. CNS signs and/or rabies negative – sample animals of any age:
    a. Diagnostic laboratories – samples submitted due to evidence of CNS clinical signs.
    b. Public health laboratories – rabies negative cases.
    c. Slaughter facilities – CNS antemortem condemns at slaughter, sampled by the Food Safety and Inspection Service (FSIS).
    d. On-the-farm – CNS FAD investigations.

3. Cattle exhibiting other signs that may be associated with BSE – Cattle that were condemned or euthanized or that died as a result of a moribund condition, tetanus, emaciation, injuries, or non-ambulatory conditions.

4. Dead cattle – Any dead cattle where the specimen is of diagnostic quality and the cause of death and/or clinical signs prior to death, if known, do not preclude it from the targeted population.

Cattle condemned on antemortem inspection at slaughter:
Samples will be collected from all cattle condemned on antemortem inspection at both State and Federally inspected slaughter plants. All cattle, regardless of age, condemned by FSIS upon antemortem inspection for CNS impairment will be sampled. All cattle, with the exception of veal calves (less than 400 pounds at slaughter), condemned by FSIS upon antemortem inspection for any reason other than CNS will be sampled. This sampling will be done on-site at the slaughter establishment unless documentable, verifiable alternative arrangements for off-site sampling have been approved in advance. Sampling done on-site at the slaughter establishment will be done by FSIS personnel at Federally inspected plants, and either APHIS or State personnel at State inspected plants. Sampling done through approved off-site arrangements will be done by APHIS personnel or their contractors. Samples will be collected from these animals and submitted to the designated laboratory regardless of sample quality. Results from testing of cattle in this category will be provided; however, unless the samples are from cattle that meet the above definition of targeted samples, these data will not be included in the statistical analysis nor will they count toward our BSE surveillance sampling target.

Collection sites:
Samples can be collected most efficiently and cost-effectively at concentration points. These are facilities where multiple animals or carcasses are collected, such as a rendering facility, a deadstock collection point, or a salvage (3D/4D) slaughter facility. While focusing efforts on these facilities will allow the highest number of samples to be collected within a defined time frame, samples may be collected at any site where targeted animals are located. In addition to those previously identified, this may include on the farm, State or Federally inspected slaughter facilities, livestock markets, veterinary clinics, diagnostic laboratories, or any other facility as necessary.

V. RANDOM SAMPLES FROM APPARENTLY NORMAL ADULT ANIMALS PRESENTED FOR SLAUGHTER

A total of 20,000 samples will be obtained from randomly selected apparently normal adult animals presented for slaughter. These animals must be greater than 30 months of age. These samples will be obtained from animals presented for slaughter in a proportional allocation at the 40 largest adult slaughter facilities that slaughter the majority of adult cows and bulls.

The samples from these selected animals may be collected either at the selected slaughter facility or at an alternative site approved in advance. Any random selection tools – either for individual facilities or for combined facilities – must be approved in advance by APHIS' Veterinary Services (VS). Carcasses from sampled animals intended for human consumption must be held until negative test results are obtained as addressed in FSIS policies (FSIS Notice, published Jan 12, 2004, Docket No. 03-048N). Although there are no requirements to hold offal or other inedible products pending test results, APHIS can assist and facilitate such storage or disposal as necessary.

Results from testing of cattle in this category will be provided; however, these data will not be included in the statistical analysis.

VI. SAMPLING AND SUBMISSION PROCEDURES FOR TARGETED SURVEILLANCE SAMPLES AND APPARENTLY NORMAL SAMPLES

The brain stem, including the obex, will be collected using a brain tissue spoon or other acceptable device. Sampling spoons and tools will be provided by NVSL to sample collectors. Fresh tissue samples will be submitted to designated laboratories as listed in the attached table.

Sample collectors are responsible for evaluating the suitability of the tissue sample. Samples that are taken from the wrong location or that are significantly autolyzed are not testable, and should not be submitted unless specific arrangements are made in advance. See attached sheet for guidance on acceptable tissue samples.

Sample collectors are responsible for accurately recording all relevant information on VS Form 10-4 and the Supplemental Data Form. Data should be entered on the electronic version of these forms – either on a tablet PC or via the web-based forms – unless such electronic entry is impossible. Hard copies of these forms should be used only for the occasional submitter or when or where the electronic system is not working. VS Form 10-4 is submitted – either electronically or in hard copy – to the designated laboratory. When submitted electronically, a complete hard copy of VS Form 10-4 does not need to accompany the shipment. A summary sheet as required by shipping regulations will suffice to accompany the shipment.

All animal identification devices, brands (via digital picture or drawing), and tattoos (refrigerate tissue containing tattoo) will be collected from each animal sampled. These identification items will be bagged, labeled with the sample number, and then attached to a copy of VS Form 10-4 and saved by the sample collector until negative results are received. In certain instances, samples from other disease programs (tuberculosis, for example) may be obtained from the same animal, and identification devices are required to accompany those samples to the laboratory. Animal identification will be recorded appropriately on all submission forms in these instances, with appropriate notification as to where the identification devices are stored.

Samples for which appropriate information is not recorded or samples from animals that do not meet the criteria listed in this memorandum will not be counted towards the target. These samples will be tested, but they will not be counted towards our surveillance sampling target.

Routine surveillance samples should be sent to the appropriate designated laboratory, according to the State in which the sample was collected. Attachment 2 provides the list of designated laboratories and the States from which they will receive samples.

Designated laboratories will report rapid screening test results to the sample submitter, the AVIC, and when requested, to plant management of the facility where the sample was collected. Rapid screening test results will be reported as either negative or inconclusive. In accordance with agreed laboratory standard operating procedures, any samples with inconclusive rapid screening test results must be immediately forwarded to NVSL for confirmatory testing.

VII. COST RECOVERY PAYMENTS

Payments for the following services may be made according to approved cost recovery guidelines and payment procedures. Additional payments may be approved on a case-by-case basis with appropriate justification provided to and approved by the Regional Office.

Refer to the Animal Health Protection Act, agreement guidance, or Federal acquisition regulations for further specific payment information.

1. Transport, including the transport of an animal or carcass to the collection site from a farm, slaughter establishment, etc., and transport from the collection site to a storage, disposal, or rendering site. Payment would be based on per loaded mile fee (for dead or live animal). In addition, appropriate local agreements with renderers or deadstock haulers can be established when routine transport requirements can be predicted.

2. Disposal for all screening test inconclusive cattle and for other sampled cattle when rendering is not an option or when the cost of transport and storage at the renderer exceeds disposal costs. This may include the cost of disposal in a landfill, equipment for on-farm burial, incineration, or alkaline digestion.

3. Storage pending test results:
    a. Cold storage as needed to maintain carcasses in acceptable condition for rendering or other processing pending test results. Storage costs may be paid per day at any facility that does not have pre-existing cold storage facilities or that has limited storage where sampled cattle carcasses or parts are held pending test results or disposal.
    b. Alternatively, a facility may choose to allow rendering to occur and hold the final product pending negative test results. In the event of a positive diagnosis, the batch and an appropriate amount of "flush" material will be purchased and disposed of rather than paying for storage of raw materials. This option may be offered to facilities that have adequate storage capacity or allow storage facilities to be built, but will require a prorated price for the product.

4. Fee-basis payments for accredited veterinarians assisting with sample collection. These payments can be made in accordance with the intent of VS Memorandum No. 534.2 and the cost recovery guidelines.

5. Sample collection. Individuals located at collection sites may be used on a per sample basis for collecting appropriate tissue samples from the targeted population. Compensation will only be provided when samples meet the criteria in this memorandum and are counted toward the targeted goal.

VIII. COMMUNICATION AND REPORTING OF TEST RESULTS

It is essential to have secure and reliable communication among individuals responsible for sample collection at collection locations, establishment managements, and NVSL or designated laboratories. The following communication guidelines will be followed:

1. Sample collector – designated laboratory communication:
   - The sample collector will notify the appropriate laboratory of incoming samples via facsimile, telephone, e-mail, or any other approved electronic method. This notification should occur even when an electronic VS Form 10-4 submission occurs. The information to be communicated will include the overnight contract delivery service tracking number, the collection site name and address, the unique submission reference number, and the number of samples. There is currently a dedicated e-mail box for notifying NVSL of incoming samples.
   - The sample collector will submit – either electronically or by hard copy - the original VS Form 10-4 and a Supplemental Data Form for each animal sampled with the samples sent to the designated laboratory. The collector will also make and distribute four copies of VS Form 10-4 (one for the collector, one for the collection site, one for the VS Area Office, and one to be maintained with the identification devices).
   - VS Form 10-4 has space to indicate the identification number for 10 animals. If additional animals are sampled, the sample collector should submit a continuation form listing the unique identification numbers for each animal. (Use VS Form 10-4A).
   - It is the responsibility of the sample collector to verify, via the overnight contract delivery service tracking system, that the submission has been delivered to the designated laboratory. If the sample does not arrive as expected, it is the responsibility of the sample collector to work with the delivery service to determine the location and delivery status of the sample. Samples not acceptable for testing will not be eligible for payment.

2. Communication from NVSL or designated laboratory:
   - The day the tests are completed, the designated laboratory will transmit a copy of the test results to the collector, the VS Area Office, and appropriate management at the collection site when requested. It is the responsibility of the collector to provide all necessary contact information on VS Form 10-4.
   - If all animals tested in the lot are rapid screening test negative, the designated laboratory will report results to the collector and, when requested, to the collection site. If any of the animals in the lot are screening test inconclusive, the designated laboratory report will be sent to the AVIC and will specify which carcasses tested inconclusive. The AVIC will notify the collection site, at the direction of the Deputy Administrator's office, in accordance with the policy on daily announcements of inconclusive results. A decision to hold or dispose of the carcasses pending confirmatory testing should be made with the concurrence of the AVIC.

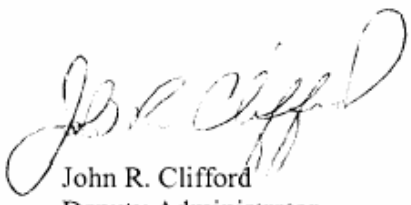
- Samples from all screening test inconclusives must be immediately forwarded to NVSL, with prior notification and confirmation of arrival. These should be sent via Federal Express or other appropriate method for overnight delivery in boxes or containers that have a security seal and in a manner that maintains chain of custody. NVSL confirmatory testing will include repeat screening tests and any other testing deemed necessary. All confirmatory test results will be transmitted directly to the VS Area Office so that carcass disposal can be coordinated and verified. The AVIC will contact the sample collector and the facility where the sample was collected.

IX. DISPOSAL

1. Disposal of carcasses and offal from screening test inconclusive or test positive animals – When necessary, disposal of carcasses and offal will be in compliance with Federal, State, and local laws. Acceptable options may include any of the following, among others:
   - Refrigerate or freeze pending test results, then render or otherwise process after negative test results are obtained (could include boning out carcass and holding the meat product for use in pet food or rendering materials and holding finished product).
   - Disposal by rendering at dedicated facilities, if available – rendering for non-animal feed use, such as biofuel or cement.
   - Burial in a landfill or on-the-farm.
   - Alkaline digestion.
   - Incineration.

2. Hides – Hides need not be disposed or held pending test results.

3. Sample disposal – Laboratories will dispose of samples using standard operating procedures.



John R. Clifford
Deputy Administrator
Veterinary Services

Attachment 1 – Personal safety

If BSE is transmissible to humans in the occupational setting, the most likely routes would be through contact with infective tissues through wounds or open lesions on the skin, contact with mucous membranes (eyes and mouth), or exceptionally, by swallowing. Transmission by the airborne route (*i.e.*, by the inhalation of infectious airborne particles) is considered to be the least likely route of exposure. In naturally BSE affected cattle, the only tissues that have shown infectivity are the brain, retina, and spinal cord. In experimentally (orally) affected cattle, the distal ileum has also shown infectivity.

Because rabies, listeriosis, and other possible zoonotic diseases must be included in the differential diagnosis, brain and spinal cord collection from cattle with central nervous system (CNS) clinical signs should be done carefully. The following precautions are generally applicable:

* Adhere to safe working practices and take extra precautions to avoid or minimize the use of tools and equipment likely to cause cuts, abrasions, or puncture wounds.
* Where use of such equipment is unavoidable, wear suitable protective clothing which includes disposable coveralls, aprons, heavy gloves and boots.
* Cover existing cuts, abrasions, and skin lesions on exposed skin with waterproof dressings.
* Use face protection such as a facemask and face shield or goggles to protect the mucous membranes of the eye, nose, and mouth from exposure to infective droplets or tissue fragments.
* Take steps to avoid the creation of aerosols and dusts when engaged in activities such as sawing through the skull bones.
* Wash hands and exposed skin before eating, drinking, smoking, taking medication, using the telephone, or going to the toilet.
* Wash and disinfect protective clothing and instruments thoroughly after use.

Attachment 2 – Designated laboratories

Designated laboratory	State in which sample collected
California Animal Health and Food Safety Lab System University of California ; Davis, CA	California, Arizona, Nevada
Colorado State University Veterinary Diagnostic Lab; Ft. Collins, CO	Colorado, Utah, Wyoming, Nebraska, South Dakota, North Dakota
Texas Veterinary Medical Diagnostic Laboratory; College Station, TX	Texas, Arkansas, Louisiana, New Mexico
Wisconsin Animal Health Laboratory; Madison, WI	Wisconsin
Washington State University Animal Disease Diagnostic Lab; Pullman, WA	Washington, Oregon, Idaho, Montana
Athens Diagnostic Laboratory, College of Veterinary Medicine University of Georgia; Athens, GA	Georgia, Mississippi, Alabama, Tennessee, Virginia, North Carolina, South Carolina, Oklahoma
NY State College of Veterinary Medicine Veterinary Diagnostic Laboratory, Cornell University; Ithaca, NY	New York, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, Vermont, Rhode Island, Delaware, Connecticut, Michigan, *Pennsylvania (western Pennsylvania only)
USDA, APHIS, National Veterinary Services Laboratory (NVSL); Ames, IA	Iowa, Illinois, Indiana, Hawaii, Alaska, Puerto Rico
Kissimee Diagnostic Laboratory, Florida Dept of Agriculture and Consumer Services; Kissimee, FL	Florida
Minnesota Veterinary Diagnostic Laboratory University of Minnesota; St. Paul, MN	Minnesota
Veterinary Diagnostic Laboratory Kansas State University; Manhattan, KS	Kansas, Missouri
U.S.Department of Agriculture Laboratory; Frankfort, KY	Kentucky, Ohio, West Virginia
Pennsylvania Veterinary Laboratory; Harrisburg, PA	Pennsylvania (central and eastern Pennsylvania)